VALIDATION OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PB-PK) MODEL USED TO SIMULATE ABSORBED MALATHION DOSES IN HUMANS

by

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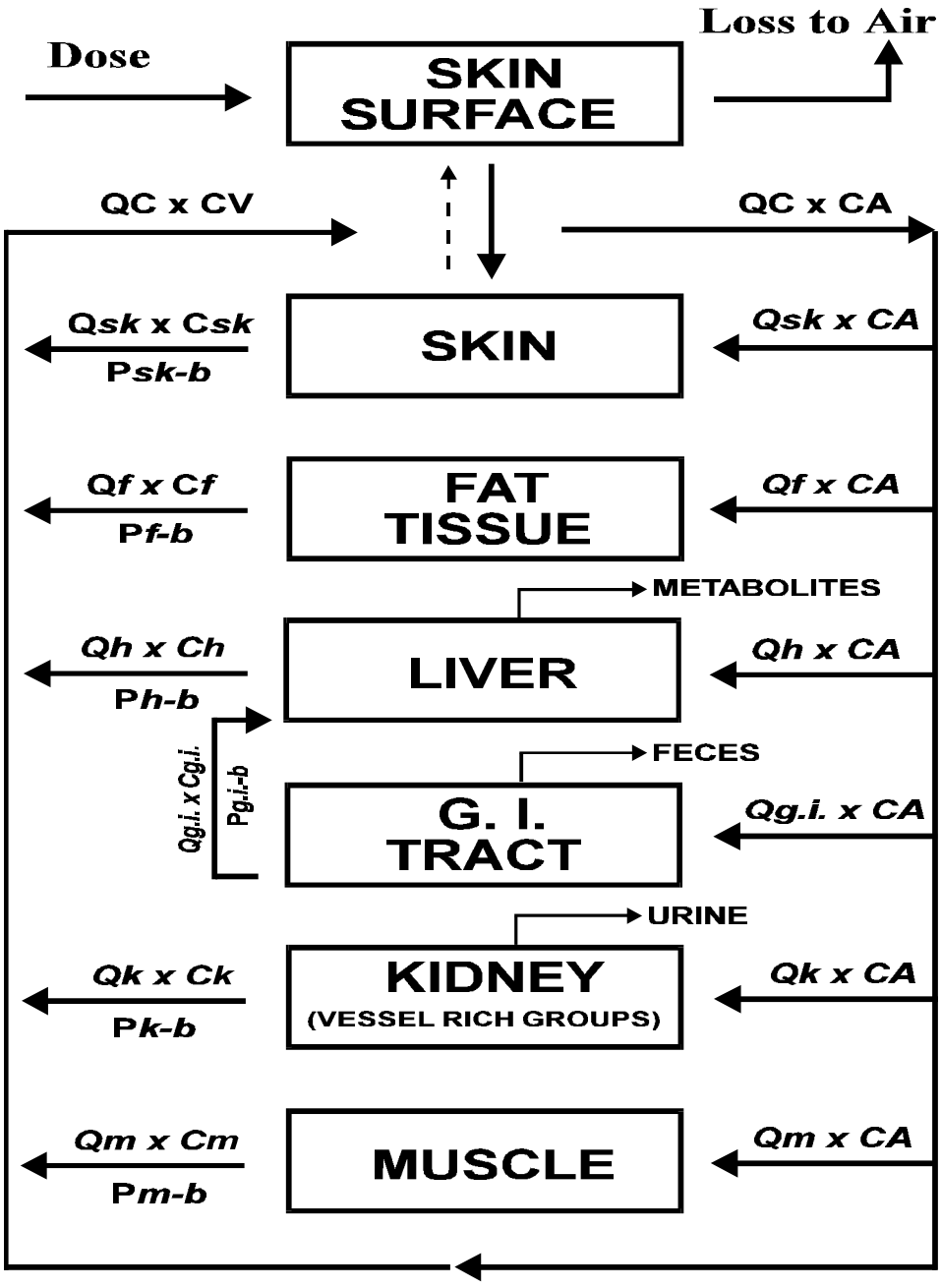
Abstract

State health and regulatory agencies in California recently have used a PB-PK model to estimate the absorbed malathion doses in 11 adults and children allegedly exposed to aerial sprays during an urban pesticide application. Simulation for those dose estimates was based on single urine samples collected within 48 h of a potential exposure. The PB-PK model used was a theoreti-cal construct at best and previously was validated with observed values from only a single volunteer. The objective of the present study thus was to further validate this model with more human data now available in the literature. The additional data were obtained from a 24-h dermal exposure study in which urine samples from 12 human volunteers were collected over a 72-h period for recovery of radiolabeled malathion. Results from the present study showed that the time-courses of the serial urinary malathion metabolites presented in the literature were highly reproducible by the PB-PK model. When urines collected at 4, 12, and 36 h after initial exposure were used as spot samples (for the 12 volunteers), as was done earlier for the 11 adults and children, the absorbed doses simulated by the PB-PK model all had a range whose upper limit was two to six times greater than the value calculated from the literature data. Such a finding was not unexpected, however, since the time-course data showed that urinary excretion of malathion and its metabolites in the volunteers had its peak around 8 - 15 h after initial exposure. It is also important to note that in many exposure situations, spot urines are the only biological samples that can be collected from humans.

Figure 1. A Typical PB-PK Model for Dermal Exposure (Reproduced from Dong *et al*. [1])



(Q*i* = blood flow to tissue *i*; P*i-b* = tissue *i* - blood partition coefficient; C*i* = concentration in tissue *i*; QC = cardiac output; CV = mixed venous concentration; CA = mixed arterial concentration; and CA ≈ CV.)

(*The mass-balance theory assures that the total absorbed dose can be calculated by summing the amounts of chemical present in the above internal tissues below the skin surface compartment; also see pharmacokinetic equations in the Appendix for specific notations used.*)

Table 1. Input Parameters Used for Modeling Dermal Absorption of Malathion[a,b]

QC = 6.475	Cardiac output (L/min)
QF = 0.2	Blood flow to fat (L/min)
QGI = 1.2	Blood flow to G. I. tract (L/min)
QK = 2.25	Blood flow to kidney (L/min)
QH = 1.5	Blood flow to liver (L/min)
QM = 1.2	Blood flow to muscle (L/min)
QSK = 0.125	Blood flow to skin (L/min)
PFC = 0.143	Percentage Fat
PGIC = 0.034	Percentage G.I. tract
PKC = 0.039	Percentage kidney
PHC = 0.021	Percentage liver (hepatic)
PMC = 0.429	Percentage muscle
PSKC = 0.039	Percentage skin
$P_{F-b} = 775.5$	Fat/blood partition coefficient
$P_{GI-b} = 15.0$	G.I. tract/blood partition coefficient
$P_{K-b} = 17.0$	Kidney/blood partition coefficient
$P_{H-b} = 33.6$	Liver/blood partition coefficient
$P_{M-b} = 22.8$	Muscle/blood partition coefficient
$P_{SK-b} = 25.0$	Skin/blood partition coefficient
$V_{max} = 4.89 \times 10^{-4}$	Michaelis-Menten rate (moles/min)
$K_m = 1.35 \times 10^{-4}$	Michaelis-Menten concentration (*M*)
$K_{sp} = 0.3 - 90 \times 10^{-5}$	Skin permeability constant (min^{-1})
$K_a = 1.0 \times 10^{-6}$	Evaporation constant (min^{-1})
$K_u = 20$	Urinary constant (min^{-1})
$K_f = 0.1$	Fecal constant (min^{-1})
BW = 70	Body weight of human volunteers (kg)

[a] incorporated into a microcomputer program written in BASICA by Dong [2]; the kinetic equations for which the computational algorithm was written are available in that report and in Dong *et al*. [1], and are *appended* here in small print for completeness only.

[b] based on those used by Cal/EPA Office of Environmental Health Hazard Assessment [3,4]; a similar list including those for children is available in Dong *et al.* [1].

Validation Procedures

1. **Available in the literature for further investigation is a recent study by Dary *et al*. [5], in which dermal absorption of neat malathion, a 50% emulsifiable concentrate (EC), and a 1% and 10% aqueous mixture of the 50% EC formulation was examined in 12 adult human volunteers. The total absorbed doses of malathion calculated from urinary radiorecoveries in these participants are listed in Table 2 below.**
2. **Figure 2 below summarizes the typical time-course of urinary excretion of malathion that can be simulated from a PB-PK human dermal exposure model that was constructed earlier by OEHHA [3,4] and later modified by Dong *et al*. [1].**
3. **To further validate its use, this PB-PK model was applied to simulate the *serial* urinary excretion of malathion observed in the 12 volunteers in Dary *et al*. [5]. The good to excellent approximation of the serial urinary outputs, as evident from Figures 3A - 3D, further suggests that a fairly good estimate for the total absorbed dose of malathion in humans can be back calculated under the mass-balance theory.**
4. **In many exposure situations, spot (*vs*. serial) urines are the only biological samples that can be collected from humans. A case in point is the recent study collaborated by several state health and regulatory agencies in California [1]. In that study, the above PB-PK model was used to simulate the absorbed malathion doses in 11 adults and children from spot urines collected after they all had complained to have some (primarily dermal) contact with the sprays during an urban aerial pesticide application.**
5. **The objective of the present study hence was to determine how (in)accurate it would be to use the PB-PK model to simulate the absorbed doses of malathion in the 12 volunteers in Dary *et al*. [5], under the pretense that only spot samples (e.g. urines collected at 4, 12, or 36 h after initial exposure) were available.**

Table 2. Absorbed Doses of Malathion Determined from Biomonitoring Data *versus* Those Simulated from PB-PK Modeling

	Biomonitoring Data			PB-PK Simulation, Spot Samples[d]			
Subject[a]	Surface[b]	Dose (mg)	% Abs[c]	0-4h	8-12h	24-36h	Average
A1	66.50	23.60	18.36	0.77	46.23	8.74	18.58
A2	27.10	24.90	2.45	0.39	8.34	2.03	3.59
A3	75.20	23.30	9.53	1.19	11.90	4.68	5.92
A4	11.70	23.70	5.05	1.56	17.10	3.58	7.41
A5	24.60	10.50	3.10	0.77	6.26	1.75	2.93
A6	29.00	23.20	5.62	1.94	16.02	1.92	6.63
B1	20.20	26.70	2.33	1.56	2.96	1.86	2.13
B2	17.00	20.40	4.24	0.39	11.04	3.91	5.11
B3	15.60	24.40	7.23	0.77	29.24	3.26	11.09
B4	24.00	13.60	9.01	2.70	24.38	4.02	10.37
B5	56.70	21.90	9.04	1.19	18.68	5.65	8.51
B6	36.80	19.80	2.57	1.19	12.04	3.87	5.70
C1	4.60	0.11	16.86	21.62	47.65	5.93	25.07
C2	4.60	0.14	19.59	14.98	49.20	10.80	24.99
C3	4.60	0.17	9.40	15.93	21.77	8.33	15.34
C4	4.60	0.12	28.60	15.93	99.9+(?)	1.05	38.99
C5	4.60	0.16	10.57	9.30	52.65	2.90	21.62
C6	4.60	0.15	11.93	20.76	8.02	3.56	10.78
D1	4.60	5.48	3.13	2.32	5.08	1.89	3.10
D2	4.60	5.50	7.48	3.83	8.47	7.25	6.52
D3	4.60	5.43	4.40	4.57	9.18	3.95	5.90
D4	4.60	5.46	4.76	3.46	13.71	3.95	7.04
D5	4.60	5.38	5.27	3.83	5.08	6.80	5.24
D6	4.60	3.98	10.99	2.70	30.93	7.95	13.86

[a] after resting for two weeks, individuals in group A that were dosed with neat malathion were given again a 1.0% aqueous mixture as members of group C; individuals in group B receiving the 50% EC formulation were dosed again with the 10.0% aqueous mixture as members of group D after resting for two weeks (*see* Dary *et al.* [5]).

[b] cm^2 of skin area in the arm, which was covered with an occlusive patch after the applied dose was observed for 4 h for spreading.

[c] cumulative percent of the applied dose of [^{14}C]-malathion recovered in the urine at 60-72 h after initial exposure.

[d] cumulative percent of the applied dose simulated to the upper end of the spot interval (i.e. 4, 12, or 36 h); the average was an arithmetic mean of the doses simulated for the three spot samples; the following exemplifies the actual amount of spot sample that was used for simulation: for 8-12h, amount = (cumulative % observed at 8-12h - cumulative % observed at 4-8h) x (12h/4h).

Figure 2. Excretion Curve for Malathion and Its Metabolites (Simulated for Subject D1 in Dary *et al*. [5])

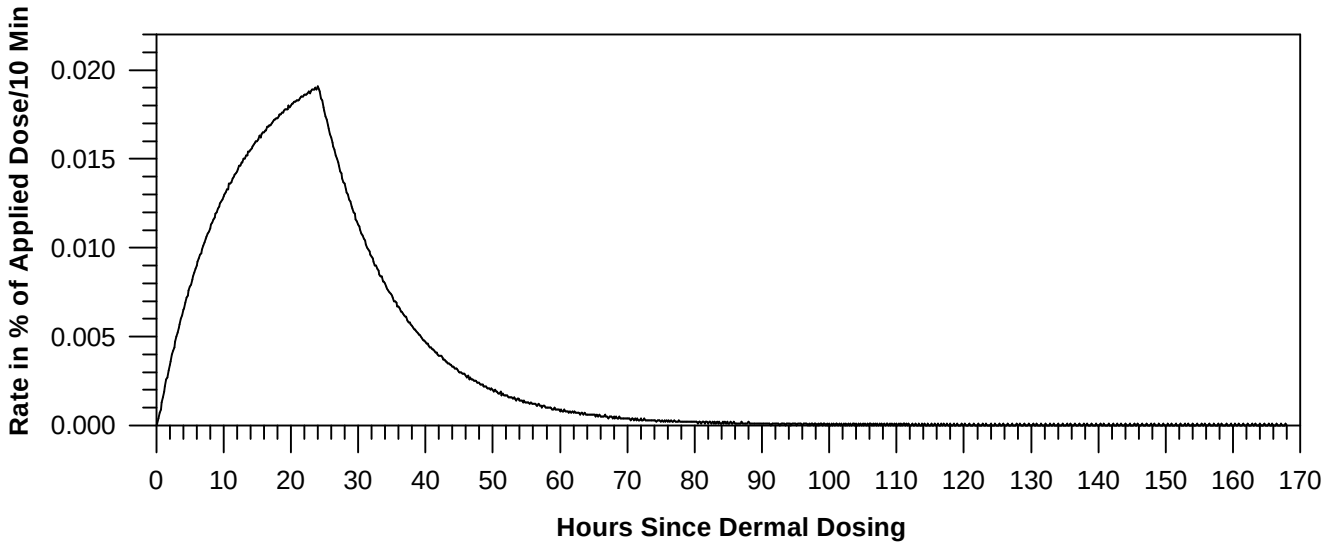


Figure 3A. Simulated vs. Observed Malathion Dose Excreted in Urine from Subject A1 in Dary *et al*. [5]

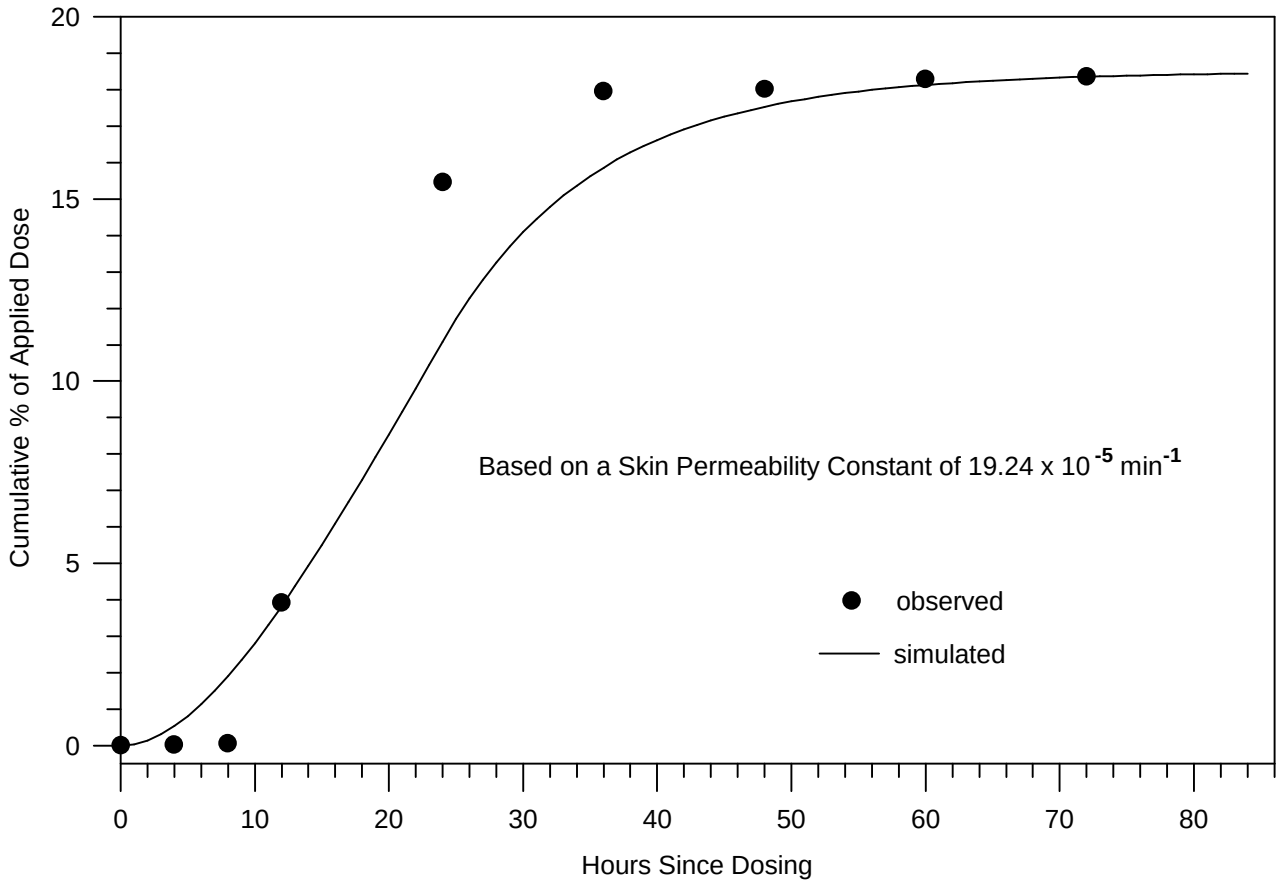


Figure 3B. Simulated vs. Observed Malathion Dose Excreted in Urine from Subject B1 in Dary *et al*. [5]

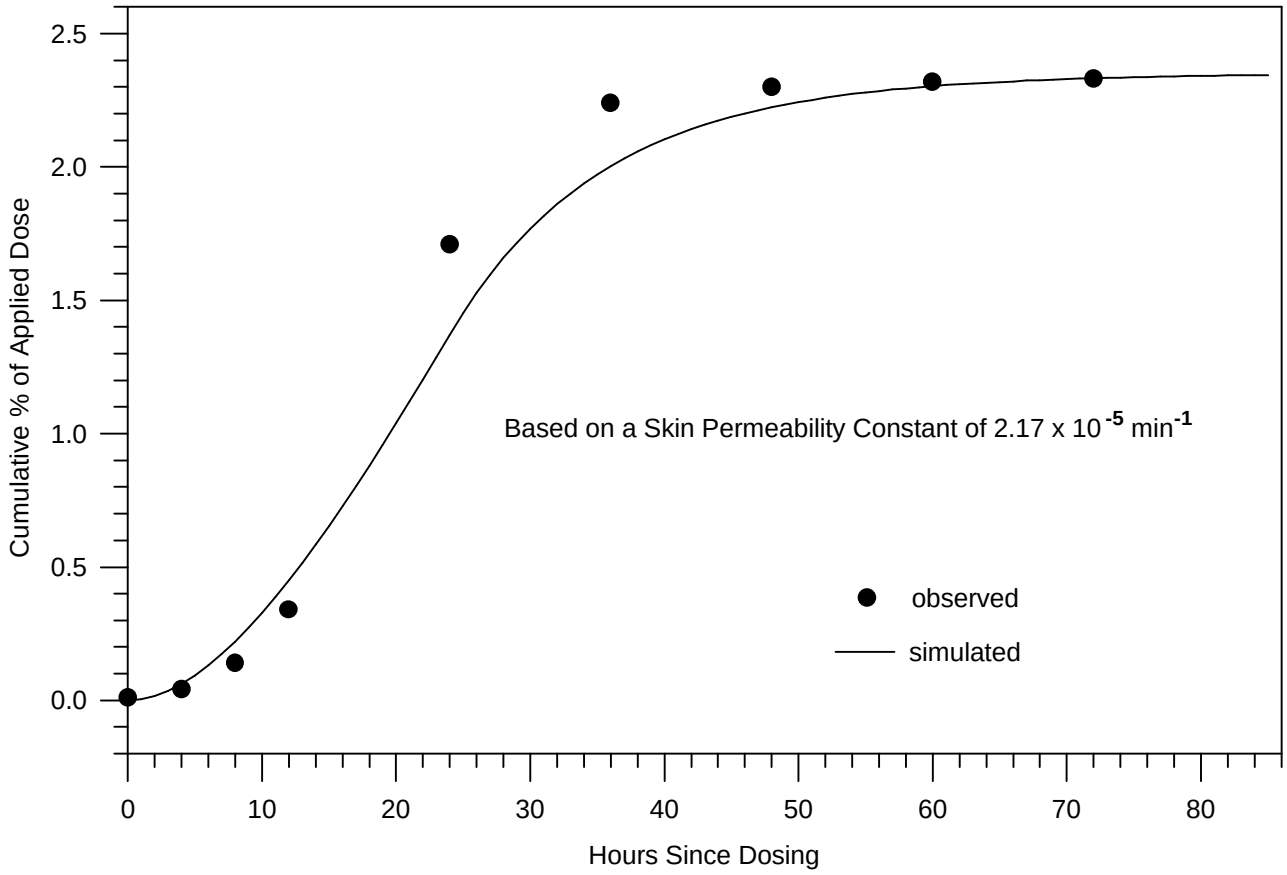


Figure 3C. Simulated vs. Observed Malathion Dose Excreted in Urine from Subject C1 in Dary *et al*. [5]

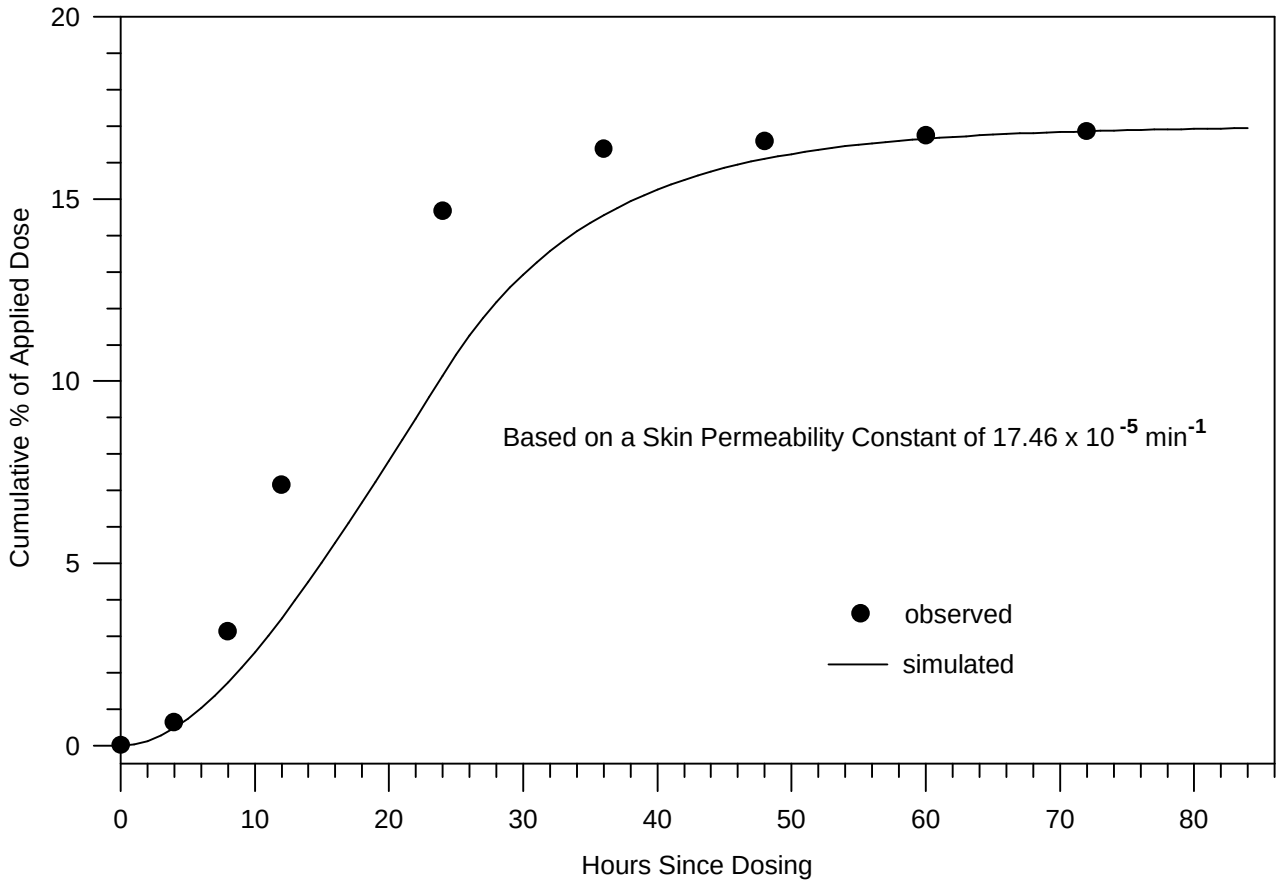
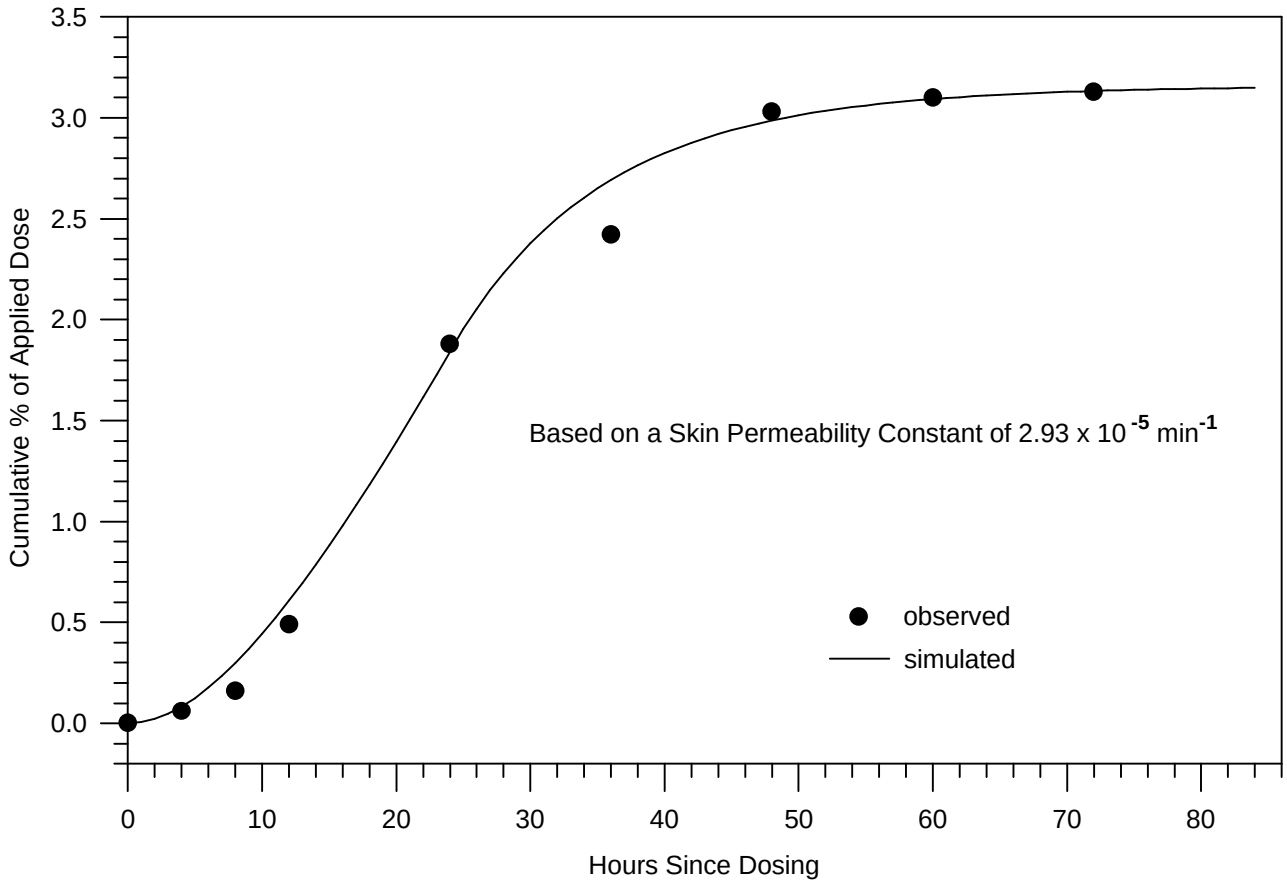


Figure 3D. Simulated vs. Observed Malathion Dose Excreted in Urine from Subject D1 in Dary *et al*. [5]



Conclusion

1. **This validation study shows that the time-courses of the serial malathion metabolites presented in human urine in the literature were highly reproducible by the PB-PK model (*see* Figures 3A - 3D). These findings were also found to be very consistent with those observed earlier in a pilot study on urines from a single volunteer [6].**

2. **The time-course data in Figure 2 show that urinary excretion of malathion and its metabolites in the 12 volunteers had its peak around 15 - 25 h after initial exposure. These observations were based on volunteers exposed to malathion dermally for 24 h. The peak for a more realistic, 8-h exposure period would occur sooner, likely around 8 - 15 h (*see* Figure 3 in Dong *et al*. [1]).**

3. **Table 2 shows that the *highest* of the three absorbed doses simulated for each of the 12 volunteers was between 1.13 (8.47/7.48) and 4.98 (52.65/10.57) times greater than the recovery value determined from biomonitoring. These simulation doses were based on spot urines collected at ≤4, 8-12, and 24-36 h after initial exposure. Thus, the highest absorbed doses from this simulation would have been likely 2 to 6 times greater if the spot urines used for simulation were collected at *exactly* 4, 12, and 36 h.**

4. **Also listed in Table 2 are the absorbed doses averaged over the three spot urines for each test subject. A difference of two-fold or less was found between these averages and their corresponding values determined from biomonitoring.**

5. **This validation study suggests that *spot* urine samples can be used to simulate the absorbed dose of malathion in humans, with an accuracy likely to be well within an order of magnitude. The accuracy could or would be improved substantially, if the simulation for each volunteer were based on two or more spot samples collected at different time points but within the first 36 - 48 h of exposure.**

References

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3. ***Health Risk Assessment of Aerial Application of Malathion-Bait*; California Environmental Protection Agency, Office of Environmental Health Hazard Assessment: Sacramento, CA, 1991. (Reprints available from Copies Unlimited, 904 Sunset Boulevard, Los Angeles, CA 90028.)**

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6. **Dong MH, Ross JH, Thongsinthusak T, Sanborn JR, Wang RG. Physiologically-based pharmacokinetic (PB-PK) model-ing for dermal absorption of pesticide (malathion) in man. *Technical Report No. HS-1678*, Worker Health and Safety Branch, California Department of Pesticide Regulation (1020 N Street, Sacramento, California, 95814).**

Disclaimer

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Appendix

Table A1. Equations Typically Used in a PB-PK Model for Dermal Exposure[a]

$$CA = \{(Q_{sk} \times C_{sk}/P_{sk\text{-}b}) + (Q_f \times C_f/P_{f\text{-}b}) + (Q_h \times C_h/P_{h\text{-}b}) + (Q_k \times C_k/P_{k\text{-}b}) + (Q_m \times C_m/P_{m\text{-}b})\}/QC \quad (1)$$

Mixed arterial (CA) concentration is the sum of the amounts eliminated in the individual compartments divided by the cardiac output QC (*i.e.*, by the sum of the individual Q_i). The amount eliminated in each compartment *i* is denoted by $Q_i \times C_i / P_{i\text{-}b}$, where Q_i = blood flow to tissue *i*, C_i = concentration in tissue *i*, and $P_{i\text{-}b}$ = tissue *i*/blood partition coefficient. (Throughout this table, the following notations are used: sk = skin; f = fat; h = hepatic; k = kidney; g.i. = GI tract; m = muscle; surf = skin surface; met = metabolite; u = urine; fec = feces; and AMT = amount.)

$$dAMT_{surf}/dt = K_{sp} \times A \times (C_{sk}/P_{sk\text{-}a} - C_{exp}) - K_a \times AMT_{surf} \quad (2)$$

The amount *on* the skin (AMT_{surf}) changes over time as a function of three events: (1) the amount diffused from *inside* to *outside* of the skin *(although at times this amount is negligible)* (2) the amount absorbed *into* the skin; and *c*) the amount lost to the air. (K_{sp} = skin permeability constant; A = surface area exposed; $P_{sk\text{-}a}$ = skin-air partition coefficient; K_a = evaporation constant; and C_{exp} = concentration of dose applied *topically.*) [Where C_{exp} is averaged *air* concentration, both this and Equation 3 will no longer be applicable since in that case the applied dose will not diminish over time.]

$$dAMT_{air}/dt = K_a \times AMT_{surf} \quad (3)$$

The amount lost to the air is a function of $K_a \times AMT_{surf}$.

$$dAMT_{sk}/dt = K_{sp} \times A \times (C_{exp} - C_{sk}/P_{sk\text{-}a}) + Q_{sk} \times (CA - C_{sk}/P_{sk\text{-}b}) \quad (4)$$

The amount absorbed into the skin is a function of three events: (1) the amount diffused *into* the skin; (2) the amount diffused from *inside* to *outside* of the skin; and (3) the amount of difference between that perfused to and that eliminated in the skin tissue.

$$dAMT_f/dt = Q_f \times (CA - C_f/P_{f\text{-}b}) \quad (5)$$

The amount in fat is related directly to the amount of difference between that perfused to and that eliminated in the fat tissue.

$$dAMT_{met}/dt = (V_{max} \times C_h)/\{(K_m \times P_{h\text{-}b}) + C_h\} \quad (6)$$

This metabolism rate is based on the well-known Michaelis-Menten equation; for some chemicals this equation may occur in a tissue organ other than the hepatic system or may take another form, such as a first-order reaction.

$$dAMT_h/dt = Q_h \times (CA - C_h/P_{h\text{-}b}) + (Q_{g.i.} \times C_{g.i.}/P_{g.i.\text{-}b}) - dAMT_{met}/dt \quad (7)$$

$$dAMT_u/dt = K_u \times AMT_k \qquad (K_u = \text{urinary constant}) \quad (8)$$

$$dAMT_k/dt = Q_k \times (CA - C_k/P_{k\text{-}b}) - K_u \times AMT_k \quad (9)$$

$$dAMT_{g.i.}/dt = Q_{g.i.} \times (CA - C_{g.i.}/P_{g.i.\text{-}b}) - K_{fec} \times GI \qquad (K_{fec} = \text{fecal constant}) \quad (10)$$

$$dAMT_{fec}/dt = K_{fec} \times GI \quad (11)$$

$$dAMT_m/dt = Q_m \times (CA - C_m/P_{m\text{-}b}) \quad (12)$$

[a] **These differential equations, reproduced from Dong *et al.* [1], are summarized graphically in Figure 1. Equations 7 through 12 are not elaborated here because on reviewing the first few equations, the reader should find their interpretations all to be repetitive. For dermal exposure, mixed venous concentration (as denoted by CV in Figure 1) is assumed to be approximately equal to CA.**